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M. N. Kirichenko, N. A. Bulychev, L. L. Chaikov, M. A. Kazaryan, A. V. Masalov, "Effect of iron oxide nanoparticles on the blood coagulation according to light scattering data," Proc. SPIE 10614, International Conference on Atomic and Molecular Pulsed Lasers XIII, 106142C (16 April 2018); doi: 10.1117/12.2303510

SPIE.

Event: XIII International Conference on Atomic and Molecular Pulsed Lasers, 2017, Tomsk, Russia

Effect of iron oxide nanoparticles on the blood coagulation according to light scattering data

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ABSTRACT

The work shows that iron oxide nanoparticles obtained in acoustoplasma discharge with cavitation affect the rate of one of the reactions of the process of blood coagulation – cleavage of fibrinogen by thrombin. As a result of the reaction, a fibrin gel is formed. By means of dynamic and static light scattering we reveal that adding of thrombin initially mixed with nanoparticles to the fibrinogen solution leads to dramatically acceleration of gel formation. Adding of nanoparticles to the solution of fibrinogen (before thrombin addition) leads to stopping of the reaction at the first stage (without gel formation). This data shows that ferrous oxide nanoparticles can act as regulators of enzyme reaction - in one case accelerating it, and in the other - by inhibiting it. Previously we presented the dynamics of distributions of the scattered light intensity on particle sizes in the fibrinogen-thrombin system with various sequences of nanoparticle addition. In this work we showed dynamics of the intensity correlation function in the samples, whose form becomes close to "stretched exponent" in pregel state or power law in a gel.

Keywords: dynamic light scattering, blood plasma, fibrinogen-thrombin reaction, iron oxide nanoparticles, acoustoplasma discharge with cavitation, intensity autocorrelation function, gel state, power law approximation.

1. INTRODUCTION

The study of transition of the sample from the liquid state to the gel one is of great interest because such a transition is one of the most important stages in complex biological mechanisms that ensure the viability and safety of the individual. So, for example, when wounding to stop bleeding in the body of higher animals and humans, a cascade of reactions of the coagulation mechanism is triggered, one of which is the fibrinogen cleavage reaction by the enzyme thrombin. As a result of this reaction, an insoluble form of fibrinogen is formed - fibrin, which forms aggregates - protofibrils, and, subsequently, a gel network. The network serves as a basis for the formation of a thrombus in the body. Thus, studies of the dynamics, rates of formation and parameters of fibrin gel are relevant both from the fundamental and the practical point of view.

At the moment, several large-scale studies of the formation of fibrin gel in vitro are presented in the literature. These are, for example, the works of Fabio Ferri et al. [1,2], who investigated the parameters of fibrin gel in a wide range of fibrinogen concentrations (with a molar thrombin to fibrinogen ratio equal to 0.01). Using a combination of two light scattering technique: light scattering at low angles and classical light scattering, they obtained dependences of the intensity of scattered light on the scattering wave vector over a wide range from $q = 3 \cdot 10^2$ up to $q = 3 \cdot 10^5$ cm^{-1}. Using the data fitting to dependences of structural model developed by them, they obtained the following characteristics of the gels: the average diameter d and the density ρ of the network fibers, the mass fractal dimensions D_m and the average size ξ of correlated blobs, which formed by entangled fibers. By varying the fibrinogen concentration c_F between 0.034–0.81 mg/ml, gels with $100>\xi>10$ mm, $100<d<200$ nm, $1.2<D_m<1.4$, and constant $\rho=0.4$ mg/ml were obtained. In their works, already formed gels were investigated, that is, the dynamics of gel formation was not investigated.

A study of the dynamics of the gel formation is carried out by Kita et al. [3], who shows the change of light scattering in samples of fibrinogen with thrombin over time. In their analysis, they rely on the change in the following parameters: the autocorrelation function of scattered light intensity, the total intensity of light scattering in the sample, and the change in

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XIII International Conference on Atomic and Molecular Pulsed Lasers, edited by Victor F. Tarasenko, Andrey M. Kabanov, Proc. of SPIE Vol. 10614, 106142C

the fracture in the Kassasa-Holtzer dependences. In addition, they also concluded on the mass fractal dimension of their gel samples and compare them to those obtained by Ferri. So, Ferri obtained the mass fractal dimension in the range of $1.2 < D_m < 1.4$, while Kita – 1.42 for a sample with thrombin concentration of 0.02 NIH/ml, and 1.51 for a sample with a concentration of 0.00125 NIH/ml. Since Ferri concentrations of thrombin were significantly higher than in Kita's work, the last one concludes that the slower the gelation process (the smaller the fibrinogen concentration), the higher the fractal mass dimension and the denser the network formed.

Returning to the intensity autocorrelation function of light scattered in the sample in the gel state, a power component appears in it according to Martin [4], which increases as the gel become more rigid. The intensity autocorrelation function for sample gel state has the following form:

$$g^{(2)}(\tau) - 1 \sim \left[A \exp(-\frac{t}{\tau_f}) + (1 - A)(1 + \frac{t}{\tau'})^{-\varphi} \right]^2, \quad (1)$$

Here, A is the factor of the light intensity scattered in diffusion mode, and τ_f and τ' are the characteristic decay time of the fast diffusive mode and the lower cutoff time of the power law behavior, respectively; φ relates to the fractal dimension of the gel structure.

In the case of a pregel state, the autocorrelation function of scattered intensity can be described as exponent stretched form:

$$g^{(2)}(\tau) - 1 \sim \left[A \exp(-\frac{t}{\tau_f}) + (1 - A) \exp\left[-(\frac{t}{\tau_s})^{\beta} \right] \right]^2, \quad (2)$$

Here, τ_s is the characteristic decay time of the slow stretched mode. Approaching the sol-gel transition point, the correlation function $g^{(2)}(\tau)$ varies from the stretched exponential form to the power-law one with β decreasing to zero and τ_s diverging.

In this paper, we present changes in the autocorrelation function in the fibrinogen-thrombin model solution at two thrombin concentrations (0.155 and 0.000155 NIH/ml), and also with the addition of new nanoparticles obtained by a unique acoustoplasma method with cavitation into the system. Investigation of the effect of nanoparticles on the gelation reaction can provide useful information for the possible introduction of these nanoparticles into medical practice. There are few works devoted to study of the effect of nanoparticles on the rate of gelation. For example, Bychkova et al.[5] shows that magnetite (Fe_3O_4) nanoparticles added to the fibrinogen-thrombin system slow down the gelling reaction in half. However, it is not indicated in which sequence the nanoparticles were added to the solution: thrombin was added to fibrinogen with nanoparticles, or thrombin with nanoparticles was added to fibrinogen. This can be of great importance, since in our works it was found out that the sequence of addition of iron oxide (Fe_2O_3) nanoparticles (obtained in acoustoplasma discharge with cavitation) dramatically affects the rate of the enzymatic reaction. In [6] we show that when nanoparticles are mixed with fibrinogen before thrombin addition, the gelation reaction stops completely, and on the contrary, when nanoparticles mixed with thrombin before its addition to fibrinogen, the reaction is accelerated. In this paper, we present the analysis of the form of autocorrelation functions obtained in the process of enzymatic reaction, corresponding to the distributions of scattered light intensity presented in [6].

The aim of the study was to investigate dynamics of autocorrelation functions in the fibrinogen-thrombin system at two thrombin concentrations, and with different sequence of iron oxide nanoparticles injection: (1) nanoparticles were added to fibrinogen solution before injection of thrombin; and (2) nanoparticles were added to thrombin solution before adding this mixture to fibrinogen.

2. METHODS AND MATERIALS

For our study we used traditional DLS setup with a He-Ne laser (the wavelength of 633 nm, the power of 5 to 10 mV), an optical system ensuring spatial coherence of recorded light, a goniometer allowing measurements at various angles, a special photomultiplier, a Photocor-FCm correlator (in the «Multiple tau» regime), and a PC with Photocor-FCm and

DynaLS programs. For a more detailed description of the setup see paper.[7] Blood plasma and protein solution samples 3 to 4 ml in volume were placed in a dust-free cylindrical cuvette 15 mm in diameter and an immersion cuvette 34 mm in diameter to ease the setup adjustment. The cuvette was thermostated at 37ºC.

Fresh blood was taken from the donor's ulnar vein to 10-ml syringes with anticoagulant K2-EDTA. The blood plasma samples were obtained by centrifuging of whole blood for 15 minutes on a lab centrifuge CLC-1 with the rate 3000 rev/min.

For model fibrinogen solution preparation we used fibrinogen from human plasma (Sigma Aldrich, ≥80% of protein is clottable) diluted in Tris-HCl buffer (7.2 pH). Final concentration of fibrinogen was about 3 mg/ml. Fibrinogen solution was filtered through 0.2 μm "Superpure PVDF" filter in order to remove essential aggregates.

Solution of thrombin was prepared using thrombin from human plasma (Sigma Aldrich, lyophilized powder, specific activity≥2,000 NIH units/mg protein) that was diluted in Tris-buffer. Final activities of thrombin in fibrinogen-thrombin solution were 0.000155 and 0.125 NIH/ml.

Iron oxide nanoparticles were obtained by unique acoustoplasma method with cavitation that combines effect of the elastic oscillations of high intensity ultrasound and pulsed or steady electric fields in a liquid medium. As a result relatively narrow size distribution of the synthesized nanoparticles with specific surface properties was obtained. Detailed description of the setup for the nanoparticles production is presented in [8, 9]. In our experiments, final volume concentration of Fe_2O_3 nanoparticles in the solution was $Cv \approx 0.5 \cdot 10^{-7}$ ($np \approx 4 \cdot 10\text{-}7\ \mu^{-3}$). Mean hydrodynamic radii of nanoparticles aggregates in the solution were of about 183 nm and 7900 nm.

3. EXPERIMENTAL

3.1 The effect of thrombin concentration on the shape of the autocorrelation function of intensity obtained in a solution of fibrinogen-thrombin

As mentioned above, during the formation of a gel in a sample of fibrinogen-thrombin (as well as some other polymers), a power-law component appears in the autocorrelation function of the intensity of scattered light (appearance of a long time tail in $g^{(2)}(t)$-1).

Figure 1 shows the change in the intensity autocorrelation function when thrombin is added to the solution at a low concentration (0.000155 NIH/ml). One can see that the experimental function obtained for pure fibrinogen (fig. 1a) is well approximated by exponential functions (red line). The function obtained after 1.5 hours for a solution of fibrinogen with thrombin in low concentration (Fig. 1b) is also fairly well approximated by the exponential dependence. However, in the experimental function "long time tail" appears, which indicates the gelation process in the sample. Approximation of the "tail" with the power law function does not give a good fitting.

Figure 2 shows the change in the intensity autocorrelation function for fibrinogen when thrombin is added to it in high concentration (0.125 NIH/ml). Fig. 2b presents that at this concentration the "long time tail" appears a lot more obvious than with a small concentration of thrombin (although it is also quite noisy). The experimental function can be approximated by the power law function with $\varphi = 0.54$, $t' = 1.73$ ms (red line). The figure also shows the approximation of the function by "stretched" exponent (blue line), but for longer times it does not work.

It should be noted that this form of autocorrelation function appears in a solution of fibrinogen - thrombin (in high concentration) within half an hour after mixing the components.

3.2 Effect of nanoparticles on the autocorrelation function of intensity in a solution of fibrinogen thrombin

It was shown in [8] that the sequence of addition of nanoparticles to the solution affects the result of the fibrinogen-thrombin reaction. So, if nanoparticles were mixed with thrombin (in high concentration) before its adding to fibrinogen, the gelation reaction is significantly accelerated. Fig. 3b shows the autocorrelation function obtained for a fibrinogen solution 15 minutes after addition of thrombin preliminary mixed with nanoparticles. One can see that the long time tail appears in the function, which is perfectly approximated by power law with the parameters $\varphi = 0{,}4$, $t' = 1{,}73$ ms (blue line). The tail is not very noisy, which, apparently, indicates that the gel is already in a denser state than in case of thrombin without nanoparticles (however, this statement requires verification). Thus, just 15 minutes after mixing of the thrombin incubated with the nanoparticles with fibrinogen, the intensity autocorrelation function shows the presence of the gel in the sample (which can be seen by the eye).

So, if fibrinogen was mixed with nanoparticles before thrombin adding to it, the gelation reaction stops almost completely. Fig. 4 shows the changes in autocorrelation function in fibrinogen solution with nanoparticles after addition of thrombin. It can be seen (Fig.4a) that experimental function for fibrinogen is well described by exponential functions, as well as fibrinogen with nanoparticles (Fig. 4b) (approximation by red lines). In addition, experimental function for fibrinogen has the "long time tail" (500 ms), which, apparently, is due to the natural process of protofibrils formation in a solution of fibrinogen. Addition of nanoparticles leads to a decrease in the contribution of this component, possibly due to the aggregation of large fibrils with nanoparticles and their precipitation. The further addition of thrombin to the fibrinogen-nanoparticle mixture results in a change in function and also the appearance of a "long time tail" contribution (Fig. 4c). However, the tail is very noisy and approximation of the function with power law (blue line) does not give very good fitting. Apparently, the process of protofibrils formation in the sample still occurs, although it is substantially slowed down.

4. CONCLUSIONS

In the paper, the study of autocorrelation intensity functions in a system of fibrinogen-thrombin with various concentrations of thrombin, as well as with a different sequence of nanoparticles addition to the solution is presented. It was found that when the concentration of thrombin is increased, the power component appears in the autocorrelation function earlier: at a low concentration after 1.5 hours (but very noisy), and at a large concentration, in half an hour (less noisy). When the iron oxide nanoparticles are incubated with thrombin, in which, apparently, its activation occurs, the power law component in the autocorrelation function appears after 15 minutes and the long time tail allows the good fitting. When adding the same nanoparticles to fibrinogen before thrombin adding to it, the function also changes ("long time tail" appears, but very noisy) and permits only a poor fitting by the power law function. This indicates that the gelation reaction is still going on, but is strongly delayed. A more detailed analysis of the dynamics of the autocorrelation functions will be presented elsewhere.

This work was partially supported by the MEPhI Academic Excellence Project (agreement with the Ministry of Education and Science of the Russian Federation of August 27, 2013, project no. 02.a03.21.0005).

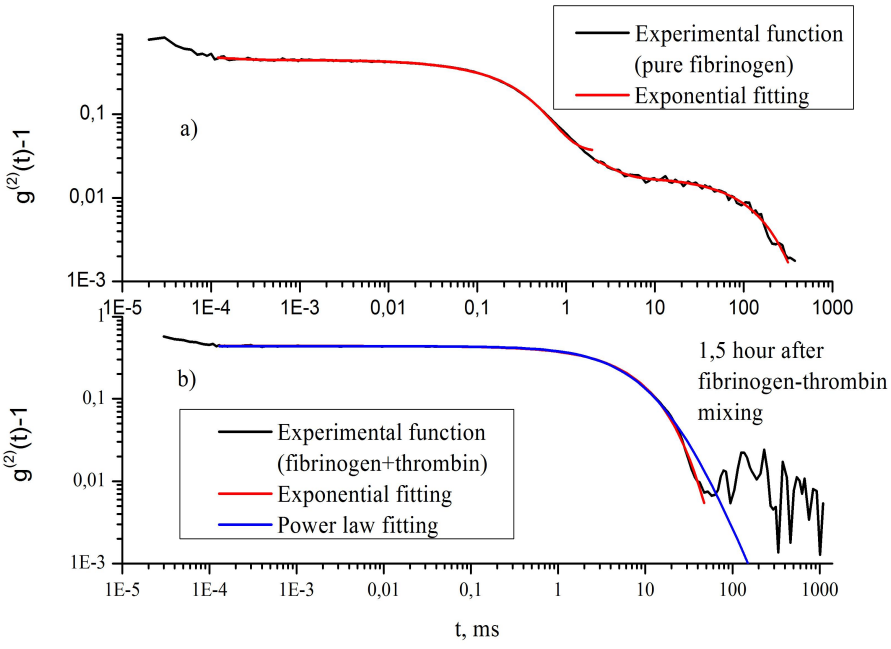

Figure 1. The autocorrelation functions of the scattered light intensity obtained for pure fibrinogen (a) and for fibrinogen with thrombin at low concentration 0.000155 NIH/ml obtained 1.5 hour after components mixing (b); red line – exponential approximation, blue line – power law approximation.

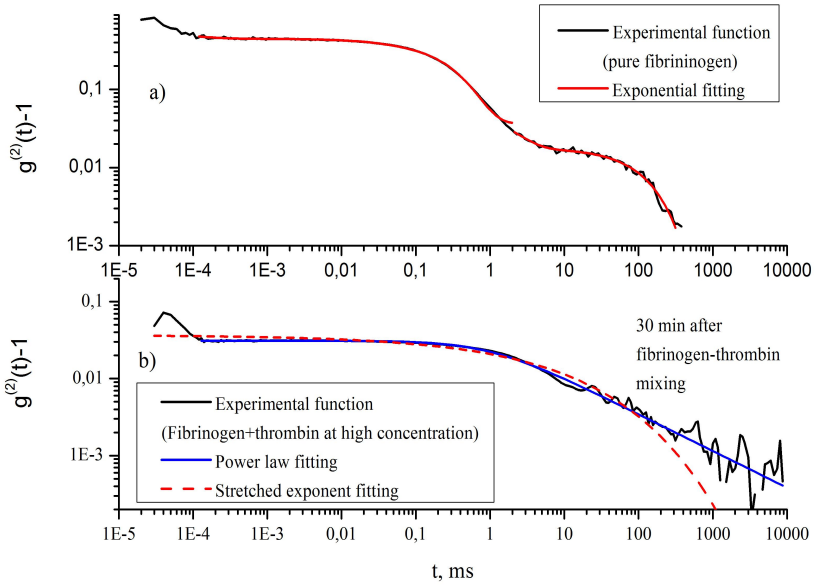

Figure 2. The autocorrelation functions of the scattered light intensity obtained for pure fibrinogen (a), and for fibrinogen with thrombin at high concentration 0.125 NIH/ml obtained 30 min after components mixing (b); red line – exponential approximation, red dashed line – "stretched" exponent, blue line – power law approximation.

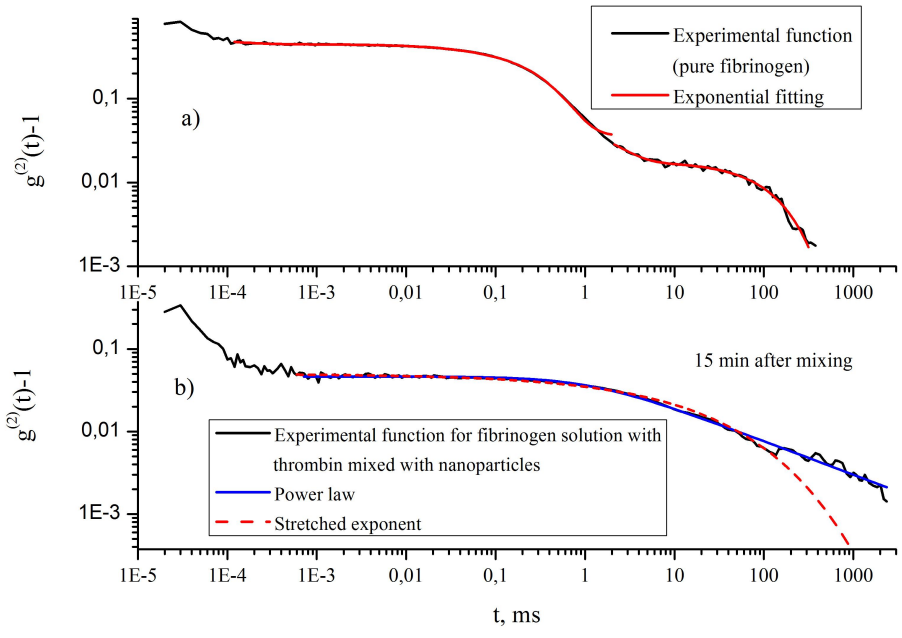

Figure 3. The autocorrelation functions of the scattered light intensity obtained for pure fibrinogen (a), and for fibrinogen solution with thrombin at high concentration 0.125 NIH/ml mixed with iron oxide nanoparticles (concentration ≈ 4·10-7 μ^{-3}); the function was obtained 15 min after components mixing (b); red line – exponential approximation, red dashed line – "stretched" exponent, blue line – power law approximation.

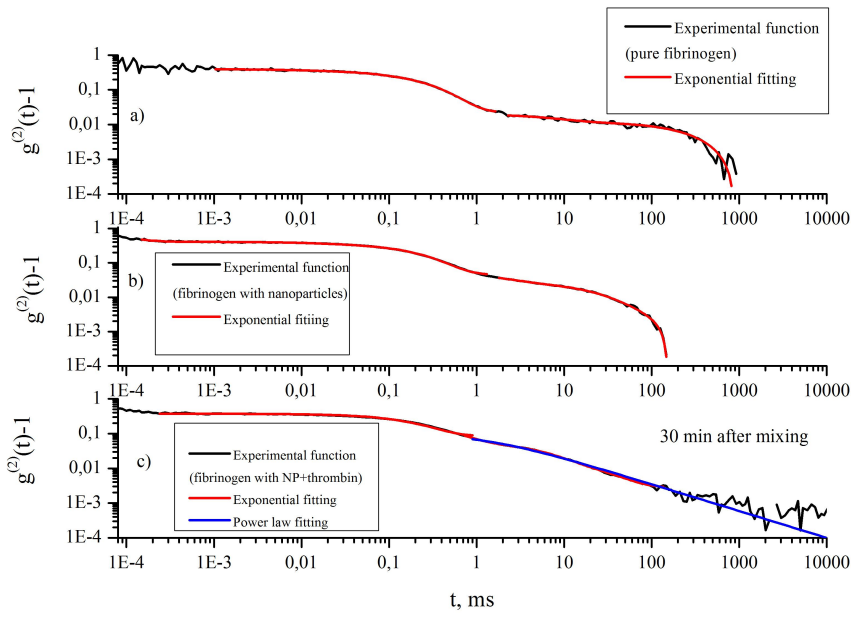

Figure 4. The autocorrelation functions of the scattered light intensity obtained for pure fibrinogen (a), for fibrinogen solution with nanoparticles (concentration ≈ 4·10-7 μ^{-3}) (b); and for fibrinogen solution with nanoparticles 30 min after thrombin addition at high concentration 0.125 NIH/ml (c); the function was obtained 15 min after components mixing (b); red line – exponential approximation, red dashed line – "stretched" exponent, blue line – power law approximation.

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